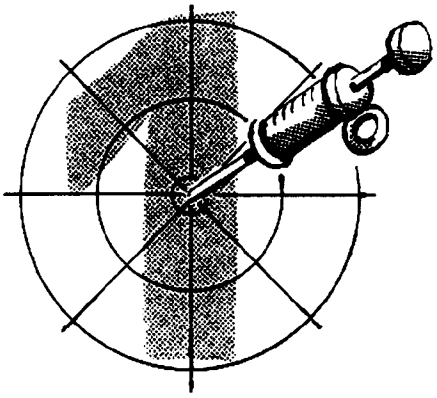
Chapter 1



INTRODUCTION

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During the past decades, angiotensin converting enzyme (ACE) inhibitors have proven their value in the treatment of hypertension and congestive heart failure. A recent application is their use in the treatment of progressive renal disease [1]. This renal protective effect is particularly reflected in two parameters: first, ACE inhibition alters the renal hemodynamics such that intraglomerular pressure falls [1]. Second, ACE inhibition effectively lowers proteinuria by 50% [2]. The latter antiproteinuric effect has been designated as a surrogate endpoint for the evaluation of the renoprotective effect of ACE inhibitors. Both effects have been postulated to be the result of local (organ or cellular) renin-angiotensin systems in the kidney. The role of the renin-angiotensin system (RAS) in pathophysiological processes and the different therapeutics that block the actions of the RAS will be discussed in a later section of this introduction.

In this thesis we describe the drug delivery of the ACE inhibitor captopril to the kidney. The renal delivery of an ACE inhibitor can be interesting for several reasons. Firstly, the renal delivery of an ACE inhibitor could clarify the contribution of local ACE inhibition to the renoprotective effects of these drugs. In the kidney, ACE is present abundantly at the brush border of renal proximal tubule and to a minor extent in the vascular endothelium [3,4]. The renal delivery of an ACE inhibitor to the kidney might be a valuable tool to study the role of this ACE in physiological and pathophysiological processes. Experiments in which the local generation of angiotensin II (Ang II) and the inhibition thereof have been studied by local administration showed the importance of the renal tissue RAS in the acute response to ACE inhibitors (vide infra).
The other reasons for which the renal delivery of an ACE inhibitor might be attractive are related to the therapeutic potential of such a preparation: Although ACE inhibitors are superior to other antihypertensive drugs in reducing proteinuria, the interindividual variation in response is considerable [2]. Therefore it will be interesting to learn if the renal delivery of an ACE inhibitor may increase the therapeutic response to this drug. Furthermore, in some patients with renal disease, for instance those without an elevated blood pressure, the maximal tolerated dose of an ACE inhibitor could be limited by the antihypertensive response. Renal delivery of an ACE inhibitor would be an interesting approach to ensure an optimal inhibition of the ACE in the kidney, possibly without the systemic effects of the drug on systemic blood pressure. Finally, renal delivery would be advantageous in that extrarenal side-effects, which can lead to the discontinuation of the treatment, are prevented by the selective action on the kidney (e.g. dry cough, angio-oedema).

In the first part of this introduction, we will describe shortly the RAS and the drugs that have been developed to block the actions of this system. Furthermore, we will review the studies in which the local renal RAS and its inhibition were investigated. In the second part, we will discuss the techniques that can be used for renal drug targeting.

THE RENIN-ANGIOTENSIN SYSTEM (RAS)

The RAS is an enzymatic system that results in the formation of Ang II, its most important bioactive component, in two consecutive steps. In the first step renin, a soluble protease, cleaves the decapeptide angiotensin I (Ang I) of angiotensinogen. In the second step, Ang I is converted to Ang II by angiotensin converting enzyme (ACE). ACE is a membrane bound protease, that is found at the luminal side of epithelial cells in the gastrointestinal tract, the renal proximal tubule and at the endothelium of vascular cells [5]. Figure 1 shows the enzymatic pathway of the RAS. In the classical view, the RAS serves as a circulating endocrine system, in which liver-derived angiotensinogen was converted to Ang I in the circulation, followed by the conversion to Ang II in the pulmonary circulation. The presence of many components of the RAS in other tissues has led to the concept of a local-acting tissue RAS [6-9]. In the latter system, Ang II is generated in the proximity of its site of action and has a paracrine and/or autocrine function. The detection of much higher concentrations of Ang II in the renal tubular fluid than in the plasma has focused the attention on a possible physiological role for the renal tissue RAS [10,11].
The circulating and tissue RAS should not be regarded as completely separate systems. In fact, the juxtaglomerular cells of the kidney serve as the major source of renin, which is distributed via the circulation to other tissues, where it is reabsorbed by mannose-6-phosphate mediated endocytosis [12]. Other components can also be supplied via the circulation to the tissue RAS [8,13]. Furthermore, since the endothelial ACE is localized at the luminal side of the vascular wall and as such generates Ang II locally in the circulation, it is often difficult to discriminate between circulating Ang II and Ang II that is generated by the tissue RAS [5,13].

Ang II exerts its actions via specific AT receptors, that belong to the G-protein coupled receptor family. So far, two AT receptors have been identified in man, and multiple subtypes of the AT_1 receptor in the rat and mouse [14]. Ang II is a potent constrictor of vascular smooth muscle cells, and induces vasoconstriction of the arteriolar blood vessels. In the kidney, arteriolar vasoconstriction reduces the renal blood flow and increases the

glomerular capillary pressure by a relative higher susceptibility of the efferent arteriole to Ang II [15]. Ang II also constricts the mesangial cells, thus reducing the glomerular filtration area [16]. Ang II stimulates sodium retention both by a direct effect on the proximal tubule and indirectly by increasing the aldosterone release of the adrenal gland [16,17].

Besides hemodynamic actions, Ang II also has trophic effects on vascular smooth muscle cells and mesangial cells [16,18]. Ang II has direct effects on cell growth, as well as indirect effects by stimulating the expression of other growth factors and an activation of the immune system [18-20]. Furthermore, Ang II has indirect effects as modulator of the activity of other vaso-active mediators, such as prostaglandins, endothelin and NO [19]. Most of the effects of Ang II are mediated by the AT_1 receptor, as was shown in studies with selective AT receptor antagonists. The biological role of the AT_2 receptor is less clear, but it might play a role in the development of organs and in tissue repair [19,21-23]. In contrast to the growth promoting effects of Ang II via the AT_1 receptor in vascular smooth muscle cells, stimulation of the AT_2 receptor at endothelial cells inhibits cell growth [24].

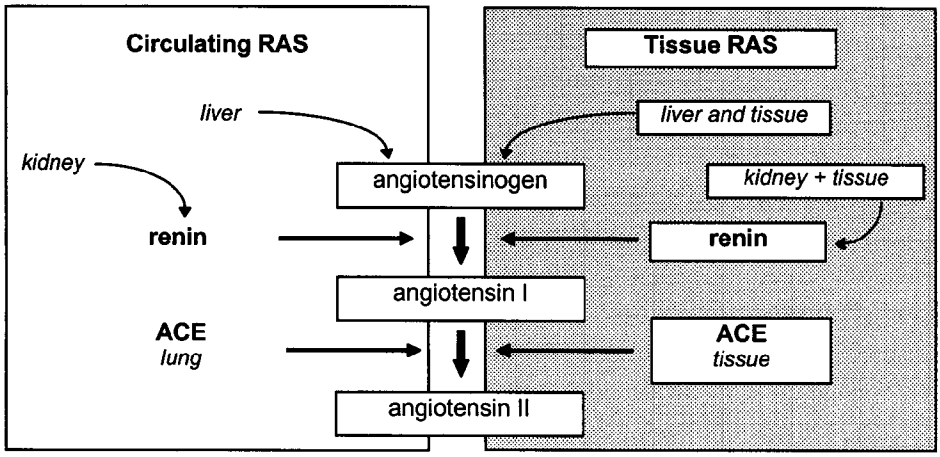

Figure 1: The renin-angiotensin system (RAS).
In the circulating RAS, liver-derived angiotensinogen is converted by kidney derived renin to angiotensin I in the circulation. Angiotensin I is converted to angiotensin II by angiotensin converting enzyme (ACE) in the lungs during the passage of the blood trough the pulmonary circulation. ACE is a membrane bound enzyme, oriented with its active side at the outside of the cell.
In the tissue RAS, the soluble components (renin, angiotensinogen or angiotensin I) of the RAS are either synthetized locally, or acquired from the circulation. Angiotensin II can be generated locally when both ACE and angiotensin I are present.

Pharmacotherapeutic intervention in the renin-angiotensin system

Two strategies have been followed in the development of drugs that intervene in the RAS, namely blocking the AT receptor with specific antagonists, and the inhibition of Ang II formation by enzyme inhibitors of renin and ACE.

AT receptor antagonists

The search for AT receptor antagonist started with the development of peptide analogues of Ang II, of which saralasin is a well-known example. Nowadays specific non-peptide receptor antagonists are available for both the AT_1 and AT_2 receptor [21,25]. The first AT_1 antagonist that was marketed for essential hypertension was losartan, and now several other AT_1 antagonists have been introduced in general practice [25,26]. Almost all of the well-known functional effects of Ang II can be blocked with the AT_1 antagonists and therefore the therapeutic potential of selective AT_2 antagonists is not evident yet. A new development is the discovery of balanced antagonists that bind to both AT receptor types. These agents may be used in order to elucidate the functional role of the AT_2 receptor [25].

Blockade of Ang II formation: renin inhibitors

The formation of Ang II can be interrupted by either inhibition of renin or ACE. Since angiotensinogen is the only known substrate for renin, the inhibition of this enzyme only has effects on the RAS. Therefore renin inhibition seems a rational approach to limit the formation of Ang II. Several renin inhibitors with a high in vitro and in vivo potency have been developed, of which remikiren and enalkiren have been investigated most thoroughly. A drawback of the developed renin inhibitors is their low oral bioavailability (<1%) and short plasma half life. Although renin inhibitors clearly decrease plasma renin activity and Ang II plasma levels, they have shown only limited efficacy in normalizing hypertension in man [27]. Whether this is due to their non-optimal pharmacokinetic profile, or for instance to an insufficient inhibition of intracellular renin, or even to other reasons, is unclear.

Blockade of Ang II formation: ACE inhibitors

In contrast to the lack of success with renin inhibitors, the development of inhibitors of ACE has been very successful. Captopril, the first non-peptide ACE inhibitor, was discovered already a decade before the development of the first non-peptide AT_1 receptor antagonist [21]. ACE inhibitors have proven to be safe therapeutics and nowadays many ACE inhibitors are registered for the treatment of essential hypertension, congestive heart

failure and renal failure [1,28,29]. ACE inhibitors reduce proteinuria and retard the chronic progression of the renal function loss in both diabetic and non-diabetic human renal disease [30-33]. In different experimental animal models of renal disease, ACE inhibitors reduced proteinuria and prevented the histological changes that were observed with the chronic progression to end-stage renal failure [34-49].

The mechanisms by which ACE inhibitors could effectuate their renoprotective effect of ACE inhibitors are summarized in table 1 [1]. Hypertension is a risk factor for the progression of renal disease and the normalization of the systemic blood pressure, either with the use of ACE inhibitors or other therapeutics, is advantageous for the conservation of the renal function. Interestingly, ACE inhibition also limits renal damage and proteinuria in a normotensive rat model of renal disease [50]. This suggests that the local effects of ACE inhibition might play an important role in the renoprotective effect of these drugs.

An important renoprotective mechanism of ACE inhibitors is their influence on the glomerular capillary pressure. Micropuncture studies showed that ACE inhibitors reduce the glomerular capillary pressure, which can be explained by a greater vasodilatation of the efferent arteriole than of the afferent arteriole [15,34,51]. The reduction of the glomerular capillary pressure has been associated with an amelioration of the glomerular permselectivity [1,33,52]. In addition, the inhibition of direct effects of Ang II on the mesangium can also lead to changes in the glomerular permeability [1].

The most apparent consequence of an improved glomerular permselectivity is the antiproteinuretic effect of ACE inhibitors [33]. ACE inhibitors reduce proteinuria by approximately 50%, and this effect is superior to other antihypertensive treatments [2]. Although first regarded as a resultant of glomerular damage, proteinuria now is considered as an independent risk factor for renal function loss [53]. Therefore the reduction in proteinuria that is conferred by ACE inhibitors might not only be symptomatic, but also related to the long-term renoprotective effect [33].

Table 1: postulated renoprotective mechanism of ACE inhibitors [1].

normalization of systemic blood pressure
control of glomerular capillary pressure
decreased mesangial macromolecular deposition
decreased mesangial cell proliferation
improved glomerular permselectivity
decreased proteinuria
decreased tubulo interstitial injury
decreased procollagen formation
improved serum-lipid profiles

Limited data are available on the renoprotective efficacy of AT_1 receptor antagonists, and it is unclear whether these agents will be equally effective to ACE inhibitors in arresting the progression of renal disease [21,33]. Besides in the formation of Ang II, ACE is also involved in the formation and inactivation of many biologically active mediators [54]. ACE inhibitors elevate local bradykinin levels and some of the cardiovascular effects and side-effects are mediated via this system [28,55]. However, AT_1 receptor antagonists share many of the therapeutic effects of ACE inhibitors, which indicates that the blocking of Ang II formation is the most important effect of ACE inhibitors [28]. No major differences have been observed between the different ACE inhibitors, although some of the effects of ACE inhibitors with a free thiol group have been attributed to the scavenging of free radicals by thiols [40,56]. Differences in tissue penetration between ACE inhibitors have been used to claim favorable effects on local RAS inhibition [28,57-60].

THE RENAL RAS AND LOCAL ACE INHIBITION

Since the kidney is a well-perfused organ, extrarenally formed Ang II can gain access to most parts of the kidney from the circulation. However, the high Ang II concentrations in the renal tissue and tubular fluid indicate that a considerable portion of the renal Ang II is locally generated [10,11,58]. Therefore it is reasonable to assume that local ACE inhibition might play a role in the renoprotective effect of ACE inhibitors [9]. Several techniques have been used to investigate the effect of local intrarenal ACE inhibition. The intrarenal formation of Ang II was studied with the intra-arterial administration of Ang I. Coadministration of a low dose of captopril diminished the depressing effect of Ang I on the renal blood flow (RBF) [61,62]. In an animal model of renal disease (uninefrectomy model), the intra arterial infusion of low doses of ACE inhibitor increased the renal blood flow (RBF) and glomerular filtration rate (GFR), decreased the renal vascular resistance (RVR), and increased the sodium excretion rate [63,64]. The combined RAS inhibition with low doses of a renin inhibitor, an ACE inhibitor and an AT receptor antagonist had an additive effect on the investigated parameters, while the intravenous administration of the compounds showed no effect [63].

A more chronic study was performed in spontaneously hypertensive rats (SHR) by the infusion of captopril into the renal medulla during 5 days [65]. Renal infusion of captopril caused an increased RBF and a negative sodium balance at 2 days after the start of the infusion. Interestingly, a gradual decrease in blood pressure was observed after intrarenal administration, but not after intravenous administration. This result indicates that, at least

in SHR, renal ACE inhibition plays an important role in the normalization of blood pressure [66].

The above described experiments show that local ACE inhibition in the kidneys can have profound effects on renal physiology and pathology but also on systemically related parameters. However, these experiments do not clarify the role of local ACE inhibition in the treatment of progressive renal disease. Many of the described studies only showed the acute effects of local renal ACE inhibition, while the beneficial effect of ACE inhibitors on the progressive renal function loss should be studied in long-term experiments.

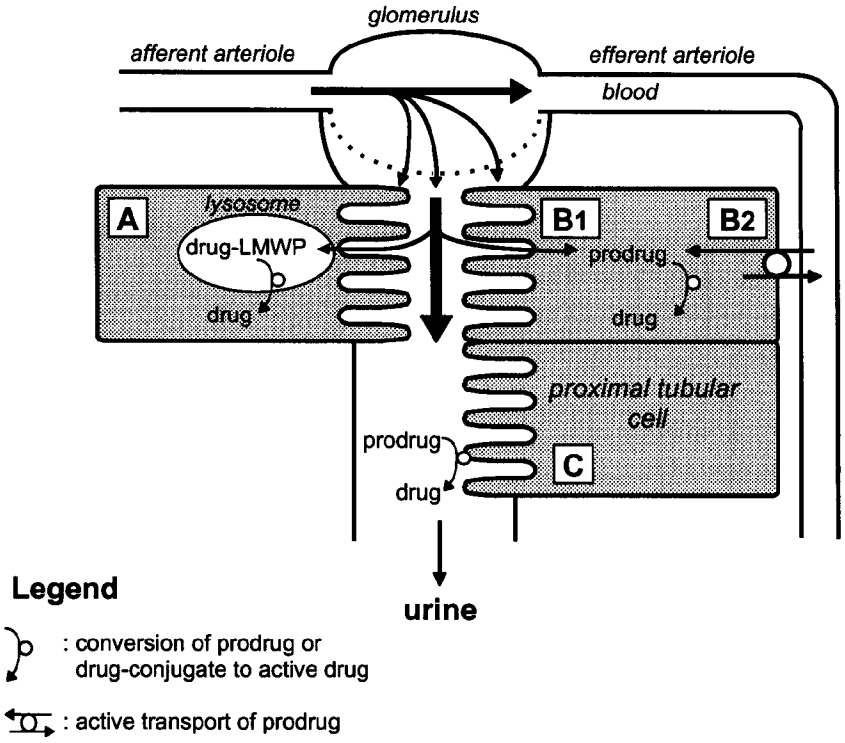

Figure 2: Strategies for drug targeting to the proximal tubular cell.
A: Drug-LMWP conjugates are accumulated at this target via adsorptive endocytosis at the luminal brush border and are degraded intralysosomally.
B: Small prodrugs can be accumulated in the proximal tubular cell via carrier-mediated uptake at the luminal brush border (**B1**) or at the basolateral side of the target cell (**B2**). This type of prodrugs can be degraded intracellularly to the active drug or, alternatively, at the brush border of the proximal tubular cell (**C**).

RENAL DRUG TARGETING

In the studies described above, the selective action of an ACE inhibitor in the kidney was achieved by an invasive route of administration: these experiments can only be performed after special surgical procedures. A selective renal action of a drug can also be attained by means of drug targeting to the kidney [67]. Drug targeting techniques aim at the manipulation of the biodistribution of drugs. By directing the distribution of the drug to a specific target, drug delivery can result in an increased effect at the target site as well as in decreased effects of the drug elsewhere in the body.

In principle, active drug targeting strategies are based on two general approaches: either selective activation or selective accumulation of the drug at the target site [68]. For both types of targeting, one has to define the specific characteristics of the target site before a successful strategy can be developed. We selected the proximal tubule as target, since the renal ACE is primarily localized at the luminal side of the proximal tubule [3,4]. The proximal tubular cell plays a major role in the elimination and reabsorption of exogenous and endogenous compounds, and these processes can be used for the renal delivery of drugs [67]. Two strategies have been applied successfully in tubular targeting: small prodrugs that are activated by site-specific enzymes, and drug-protein conjugates that are actively accumulated in the proximal tubular cell. Figure 2 shows a schematic representation of the proximal tubular cell, and the different routes by which small prodrugs and drug-protein conjugates are accumulated and activated in this cell. Both drug targeting concepts will be discussed briefly.

Prodrugs

Prodrugs are inactive substances that are converted to the active parent compound in vivo [68]. If the activation process is unique for the target site, the prodrug will be activated site-selectively. The design of kidney-selective prodrugs was based upon the relative higher amounts of some enzymes in the proximal tubular cell than elsewhere in the body. These strategies either aim at enzymes that are expressed at the brush border of the proximal tubule, such as γ-glutamyltranspeptidase (γ-GGT), or at cytosolic enzymes [69-77]. Despite the relative excess of these enzymes in the kidney, not all of the developed prodrugs showed selectivity for the kidney [70,73,78]. The renal selectivity depended often on the carrier-mediated uptake of the prodrug into the proximal tubular cell, which was mediated by the organic acid carrier [73,75]. Since this is actually a secretory pathway, a large proportion of the prodrugs was excreted intact into the urine [73].

Low molecular weight proteins

The second strategy for drug delivery to the proximal tubular cell is aimed at the reabsorption process for proteins. In the glomerulus, the blood is ultrafiltered through the glomerular basal membrane. This membrane is a barrier for large proteins, but molecules smaller than albumin (64 kD) can pass the filter [79,80]. In the proximal tubule, the filtered proteins are reabsorbed by receptor mediated endocytosis, a process that is mediated by the megalin/gp330 receptor [81]. The endocytic vesicles are routed to the lysosomal compartment, where the proteins are degraded to peptides and amino acids [79,82,83]. This pathway is the major degradation route for the so-called low molecular weight proteins (LMWPs, smaller than 20 kD). It has been demonstrated that these proteins accumulate selectively and rapidly in the kidney [84,85]. For these reasons, LMWPs qualify as potential drug carriers for renal drug targeting [67,86]. Drug-LMWP conjugates, in which the drug is attached covalently to the protein, will finally accumulate in the lysosomal compartment of the proximal tubular cells. If the linkage between drug and LMWP has been designed in such a way that it is susceptible to the lysosomal enzymes, or to the acidic pH of this compartment, the drug will be released from the conjugate in this compartment [87].

As can be concluded from the discussed strategies above, drug targeting to the proximal tubular cell can be carried out with both small prodrugs and drug-LMWP conjugates. In the present investigation we used the latter technique for the renal delivery of an ACE inhibitor. We prefer the approach with LMWP-conjugates, since the selective accumulation of the drug in the proximal tubular cell is less dependent on the specific physicochemical properties of the drug itself than when small prodrugs are used. The prodrug strategies aim at specific enzymes, and only a limited number of drugs will yield good prodrug substrates for these enzymes. The investigated prodrugs for renal drug delivery were prepared from drugs that contained either a primary amino group (γ-glutamyl and N-acetyl-γ-glutamyl prodrugs), an aromatic thiol group (β-lyase prodrug) or phenolic hydroxyl groups (alcalic phosphatase (prodrugs). With respect to the ACE inhibitors, only lisinopril would meet the requirements for the preparation of such a prodrug. On the other hand, the LMWP concept can be used for the renal delivery of both hydrophilic and lipophilic drugs. During the past 10 years, we have investigated renal drug targeting using the LMWP lysozyme (14 kD) as drug carrier [86,88]. These experiments demonstrated the importance of the biodegradability of the linkage between the drug and LMWP [89]. Therefore we reviewed various approaches to conjugate an ACE inhibitor to an LMWP via a biodegradable linkage, as will be discussed in chapter 2.

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